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REC'D 29 APR 1996
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 16865-0174	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US95/01197	International filing date *(day/month/year)* 27 JANUARY 1995	Priority date *(day/month/year)* 31 JANUARY 1994
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant TRUSTEES OF BOSTON UNIVERSITY		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

- I [X] Basis of the report
- II [ ] Priority
- III [ ] Non-establishment of report with regard to novelty, inventive step or industrial applicability
- IV [ ] Lack of unity of invention
- V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI [ ] Certain documents cited
- VII [ ] Certain defects in the international application
- VIII [X] Certain observations on the international application

C. VAN AMSTEL 04. 9. 1996
ø for S.R

Date of submission of the demand	Date of completion of this report
24 JULY 1995	16 APRIL 1996
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer SCOTT D. PRIEBE Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet) (January 1994)*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US95/01197

I. Basis of the report

1. This report has been drawn on the basis of *(Substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments)*:

[X] the international application as originally filed.

[X] the description, pages 1-66 , as originally filed.
pages NONE , filed with the demand.
pages NONE , filed with the letter of _____.
pages _____ , filed with the letter of _____.

[X] the claims, Nos. 1-103 , as originally filed.
Nos. NONE , as amended under Article 19.
Nos. NONE , filed with the demand.
Nos. NONE , filed with the letter of _____.
Nos. _____ , filed with the letter of _____.

[X] the drawings, sheets/~~fig~~ 1-14 , as originally filed.
sheets/~~fig~~ NONE , filed with the demand.
sheets/~~fig~~ NONE , filed with the letter of _____.
sheets/~~fig~~ _____ , filed with the letter of _____.

2. The amendments have resulted in the cancellation of:

[X] the description, pages NONE.

[X] the claims, Nos. NONE.

[X] the drawings, sheets/~~fig~~ NONE.

3. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the ~~Supplemental Box~~ Additional observations below (Rule 70.2(c)).

4. Additional observations, if necessary:

NONE

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US95/01197

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims	1-85, 92-103	YES
	Claims	86-91	NO
Inventive Step (IS)	Claims	1-85, 92	YES
	Claims	86-91, 93-103	NO
Industrial Applicability (IA)	Claims	1-103	YES
	Claims	NONE	NO

2. CITATIONS AND EXPLANATIONS

(See Supplemental Sheet.)

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claims 1-29, 38-85, and 93-103 are objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claims are indefinite for the following reason(s):

Claims 1-29 and 38-85 are indefinite for recitation of "capable of producing" in step b) of the base claims 1, 38, 50, 73, and 79. As written, it is unclear if the cell population produces antibodies or receptor proteins or, if they do, under what conditions and whether the antibodies or receptor proteins are produced in response to the biological sample.

Claims 1-29 and 38-72 are indefinite for omitting essential method steps. Step h) of claims 1 and 50 and step f) of claim 38 recite selection of a subpopulation of vectors. The description describes the selection of display phage, not expression vectors, by a series of adsorption steps onto normal and neoplastic tissues. The steps by which the selection process is carried out should be recited.

Claim 12 is unclear. How can the V_H and V_L cDNAs be cloned at a restriction site in the hybridized complementary sequence which links them?

Claims 13-15, 45, 56, 74, 84, and 87 are indefinite for recitation of "phages". Phage are not vectors.

Claims 69-70 are indefinite for recitation of "a biological sample" in claim 69. It is not clear whether or not "a biological sample" in claim 69 is the same "biological sample" recited in claim 50.

Claims 93-103 are indefinite because step a) in claim 93 is unclear. As written, it is not clear what transcription units are head-to-head. The step can be interpreted as inserting a single fragment in a head-to-head transcriptional orientation relative to a transcription unit present on the vector or two fragments in a head-to-head transcriptional orientation.

Claims 93-103 are indefinite for recitation of "reinserting" in step c) of claim 93 and "reinserted" in claim 100. Since the fragments were not removed from the second vector, they cannot be reinserted into it.

Claims 93-103 are indefinite for recitation of "significant loss" in claim 93. "[S]ignificant loss" has not been defined. Since the term "significant" is subjective in this context, the term is vague.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

CLASSIFICATION:

The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(6): A61K 51/10, 35/14; C12P 21/00, 21/08; C12N 15/13; C07K 16/30 and US Cl.: 424/1.49, 134.1, 138.1; 435/69.1, 172.3, 320.1; 530/387.1, 389.1

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):

Claims 86-91 lack novelty under PCT Article 33(2) as being anticipated by Hogrefe et al.

The claims are drawn to expression vectors which comprise restriction sites for insertion of fragments in a head-to-head transcriptional orientation, transcription control sequences, such as an RNA polymerase initiation site, translation control sequences, such as a ribosome binding site or a leader sequence. The vectors can be prokaryotic phagemids, cosmids, surface display vectors, or combinations thereof. It is noted that restriction sites can be used for insertion of fragments regardless of their transcriptional orientation.

Hogrefe et al. disclose ImmunoZap vectors which meet the claim limitations (Fig. 3, page 123).

Claims 93, 95, 97, 99-101, and 103 lack an inventive step under PCT Article 33(3) as being obvious over Hogrefe et al. The claims are drawn to methods of transferring a library of cloned fragments from a first vector to a second vector without significant loss of diversity (claim 93). The first vector can be a prokaryotic expression vector (claim 95). Insertion of fragments into the first or second vector can be by ligation claims 97 and 100). The fragments can be recovered from the first vector using PCR (claim 99). The starting library can comprise at least 100 different fragments (claim 101). The transfer results in a loss of diversity of less than 50% (claim 103).

Hogrefe et al. disclose a method of transferring combinatorial antibody libraries of fragments between different prokaryotic expression vectors using PCR to obtain the fragments. Ligation was used to insert the fragments into the second vector, a phage display vector (Fig. 2, page 122; Fig. 3, page 123) without any significant loss of diversity (page 121; Table I, page 123; page 126). The results in Table I suggest that the library contained at least 100 members since the binding activity assayed made up less than 1% of the library (page 123) and the reference describes the desirability of being able to access libraries of greater than 10^7 clones (page 119).

The method differs from the instant invention only in the arrangemer of transcription units within the fragment being inserted into the second vector. It would have been obvious to one of ordinary skill in the art at the time the invention was made that the PCR method used by Hogrefe et al. to obtain the fragments from the first vector prior to transfer would be suitable for any arrangement of sub-fragments with a reasonable expectation of success. One would have been motivated to use the method of Hogrefe et al. because of its efficiency and to allow expression of the antibodies on the surface of phage.

Claims 94 and 96 lack an inventive step under PCT Article 33(3) as being obvious over Hogrefe et al. in view of Orlandi et al. Claim 93 is further limited by claim 94 to libraries of cDNA fragments, and by claim 96 to second vectors that are eukaryotic expression vectors.

Hogrefe et al. has been described. Hogrefe et al. do not disclose the source of the fragments in the library or transfer to a eukaryotic expression vector. Orlandi et al. discuss cloning immunoglobulin genes from cDNA or genomic DNA as a source of variable domains for subcloning (page 3833) into eukaryotic expression vectors to generate chimeric antibody genes for subsequent expression of the antibodies from mammalian cells (page 3835, Fig. 2; page 3836, Fig. 5).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to use cDNA fragments, as generally taught by Orlandi et al. to generate the library of Hogrefe et al. with a reasonable expectation of success since cDNA was an art recognized source of gene fragments. It would also have been obvious to transfer the library fragments of Hogrefe et al. using the PCR method into eukaryotic expression vectors for the expression of cloned chimeric antibodies from mammalian cells, as taught by Orlandi et al.

Claim 98 lacks an inventive step under PCT Article 33(3) as being obvious over Hogrefe et al. in view of Swaroop et al. Claim 93 is further limited by claim 98 to excision of the fragments from the first vector using restriction endonucleases. Hogrefe et al. has been described; the reference does not teach using restriction endonucleases to release the library fragments from the first vector. Swaroop et al. teach an efficient method of transferring cDNA libraries between vectors wherein the fragments are cleaved from the first vector using the NotI restriction endonuclease.

It would have been obvious to one of ordinary skill in the art at the time the invention was made that

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

either restriction er onucleases or PCR could be used to obtain fragments from a first vector for transfer to a second vector with a reasonable expectation of success. One would have been motivated to use restriction endonucleases, such as NotI, to effect transfer of the fragments between vectors designed to accept NotI fragments, as taught by Swaroop et al.

Claim 102 lacks an inventive step under PCT Article 33(3) as being obvious over Hogrefe et al. in view of Clackson et al. Claim 93 is further limited by claim 102 to libraries of at least 10^8 different members.

Hogrefe et al. have been described, the reference does not disclose the size of the starting library used. Hogrefe et al. disclose that the cloning efficiency of their method was in excess of 10^8 pfu per microgram of DNA (page 121). Clackson et al. disclose generating cloned combinatorial "antibody" libraries of greater than 10^7 members for the purpose of mimicking features of immune selection and to increase the coverage of epitope recognition and of clones displaying the highest binding affinity for given epitopes (pages 625 and 627).

It would have been obvious at the time the invention was made that libraries of greater than 10^8 members could be generated with a reasonable expectation of success, as taught by Clackson et al., and that the methods of Hogrefe et al. had sufficient cloning efficiency to allow transfer of such libraries between vectors with a reasonable expectation of success. One would have been motivated to use the libraries of Clackson et al. in the method of Hogrefe et al. because the libraries increased the coverage of epitope recognition and of clones displaying the highest binding affinity, as taught by Clackson et al.

Claims 1-85 and 92 meet the criteria set out in PCT Article 33(2)-(4), because the prior art does not teach or fairly suggest a method of generating populations of antibodies specific for a given biological sample, such as neoplastic tissue, comprising induction of an immune response to the biological sample in a cell population capable of producing antibodies, generating cDNAs from V_H and V_L mRNAs from the cell population, amplifying and linking the V_H and V_L cDNAs in a head-to-head orientation, amplifying the linked sequences to create a population of DNA fragments encoding both V_H and V_L antibody fragments and cloning the fragments into expression vectors. The prior art also does not teach or fairly suggest the expression vectors claimed.

---------- NEW CITATIONS ----------

Gene, Vol. 128, issued 1993, Hogrefe et al, "A bacteriophage lambda vector for the cloning and expression of immunoglobulin Fab fragments on the surface of filamentous phage", pages 119-126, see entire document, especially pages 119 , 121-123, and 126.

Proceedings of the National Academy of Sciences, USA, Vol. 86, issued May 1989, Orlandi et al, "Cloning immunoglobulin variable domains for expression by the polymerase chain reaction", pages 3833-3837, see pages 3833, 3835-3836.

Nucleic Acids Research, Vol. 16, No. 17, issued 1988, Swaroop et al, "Charon BS (+) and (-), versatile lamba phage vectors for constructing directional cDNA libraries and their efficient transfer to plasmids", page 8739.

Nature, Vol. 352, issued 15 August 1991, Clackson et al., "Making antibody fragments using phage display libraries", pages 624-628, see pages 625 and 627.